CORRELATION BETWEEN OXIDATION AND PHOSPHORYLATION IN MITOCHONDRIA OF THE GASTRIC MUCOSA

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The state of cell respiration and oxidative phosphorylation in the mitochondria of the gastric mucosa was studied at different phases of gastric function. Stimulation of secretion by histamine was shown to increase the rate of mitochondrial respiration while at the same time considerably reducing the coefficients of coupling, including P/O. The above changes in the degree of energy coupling of the mitochondria of the parietal cells preceded activation of HCl secretion.

KEY WORDS: mitochondria; parietal cells; histamine; secretion of hydrochloric acid; free oxidation reactions.

The process of generation of H^+ ions by the parietal cells during hydrochloric acid secretion is closely bound with mitochondrial oxidation reactions [3, 6, 12]. Regulation of the degree of coupling of oxidation and phosphorylation is a factor in the intensification of cellular activity and it explains the phenomenon of "hypermetabolism" [7, 8, 10, 11].

This paper describes the results of a study of the intensity of respiration and its coupling with phosphorylation reactions in the mitochondria of the gastric mucosa before and after histamine stimulation.

EXPERIMENTAL METHOD

Mitochondria of the gastric mucosa of the experimental animals (albino rats) were isolated by differential centrifugation, using the following isolation medium: 0.25 M sucrose, 0.001 M EDTA, and 0.02 M Tris-HCl buffer, pH 7 4. The O_2 uptake of the mitochondria was recorded polarographically [5]. The composition of the incubation medium in the polarographic cell was as follows (in mM): sodium succinate 10, KCl 75, $MgCl_2$ 5, KH_2PO_4 20, EDTA 1, and Tris-HCl buffer, pH 7.4, 20. The following coupling coefficients were calculated: the P/O ratio, the respiratory control (RC) as defined by chance, and the reaction of the mitochondria to 2,4-dinitrophenol (DNP). The rates of O_2 consumption (calculated per 10 mg mitochondrial protein) with the respiration substrate (V_1), in the presence of ADP (V_3) as phosphate acceptor and after its exhaustion (V_4), and after the addition of DNP (V_{DNP}) were determined. The ADP was added in a concentration of 0.1 M, DNP from 0.02 to 0.04 mM, and histamine $1 \cdot 10^{-5}$ and $1 \cdot 10^{-4}$ M. In the experiments in vivo histamine was injected subcutaneously in doses of 0.01 and 0.04 mg/kg. Gastric juice was aspirated through a nasal gastric tube under conditions of basal secretion and after histamine stimulation. Phosphorus was determined by the method of Lowry and Lopez in Skulachev's modification [8] and protein by Lowry's method [15].

EXPERIMENTAL RESULTS AND DISCUSSION

According to the results in Table 1, under basal gastric secretion conditions (without histamine) the P/O ratio was 1.77, evidence of a low level of coupling of oxidation and phosphorylation. The same conclusion could be drawn from the very slight reaction of the mitochondria through addition of ADP (V_3) and DNP (V_{DNP}). Stimulation of secretion by histamine more than quadrupled the rate of O_2 consumption and caused a definite decrease in the coupling coefficients. With such a high level of respiration, the decrease in the P/O ratio was

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TABLE 1. Effect of Histamine on Intensity of Respiration and Degree of Coupling with Phosphorylation Reactions in Mitochondria in Biopsy Specimens of Gastric Mucosa (M ± m)

Experimental conditions	O_2 utilized (in μatoms/10 mg protein/min)				Coefficient of coupling				P/O
	V_1 respiration substrate	V_3 inorganic phosphate acceptor ADP	V_4 exhaust. of phosphate acceptor ADP	V_{DNP}	V_3/V_4	V_3/V_1	V_{DNP}/V_3	V_{DNP}/V_2	
Control	6,44±0,71	8,97±0,94	6,84±0,76	12,98±1,94	1,31±0,20	1,4±0,18	2,5±0,17	2,0±0,23	1,77±0,21
Histamine (0.04 mg/kg, subcutaneous inject.)	25,25±2,29*	12,03±1,11*	15,89±1,76*	20,13±2,84*	0,7±0,18*	0,48±0,14*	1,66±0,12*	0,8±0,098*	0,84±0,081*

*$P < 0.05$ compared with control.

Legend. Mean values from 11 experiments in control and experimental series.

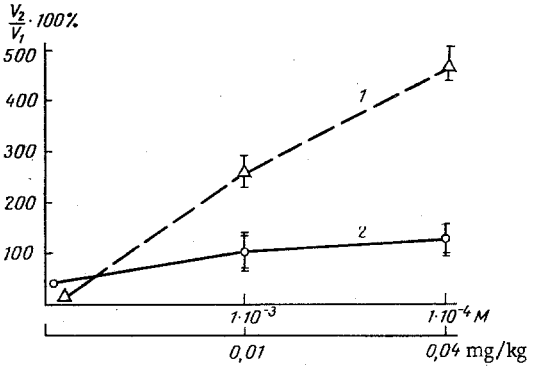

Fig. 1. Effect of histamine on intensity of cell respiration of mitochondria of gastric mucosa. Abscissa, concentrations and doses of histamine; ordinate, ratio of V_2 (rate of O_2 utilization after treatment with histamine) to V_1 (rate of oxygen utilization in presence of respiration substrate), in %. 1) Intensity of respiration after injections of histamine; 2) intensity of respiration after direct addition of histamine to incubation medium.

50% of its initial value, confirming the high degree of activation of free oxidation. To discover the mechanisms of the uncoupling effect thus observed, the rate of neutralization of O_2 by the mitochondria was studied before and after the action of histamine, when administered by different methods. In the first case histamine was given by subcutaneous injections 30 min before subsequent decapitation of the animals and removal of their gastric mucosa; in the second case histamine in different concentrations was added directly to the incubation medium, and incubation also continued for 30 min. The experimental results (Fig. 1) showed that histamine has no uncoupling action in vitro. The increased O_2 demand in the presence of histamine in a concentration of $1 \cdot 10^{-5}$ M was negligible and the increase in the ratio of respiration with a higher concentration ($1 \cdot 10^{-4}$ M) was not statistically significant. Conversely, after subcutaneous injection of histamine marked intensification of mitochondrial respiration correlating with the size of the dose of histamine was observed. These results prove the indirect character of the uncoupling action of histamine, through its effect on the adenylate cyclase–cyclic AMP system of the parietal cells. According to the writers' views [4] on the mechanism of action of histamine on gastric secretion, the stimulation of lipolysis by cyclic AMP leads to a rapid increase in the concentration of fatty acids, highly important metabolic substrates and, at the same time, regulators of the degree of energy coupling of mitochondrial oxidation reactions [9, 14]. In turn, the study of the correlation between oxidation and phosphorylation in the gastric mucosa during histamine-stimulated acid formation (Fig. 2) showed that active HCl production is preceded by a marked increase in the intensity of mitochondrial respiration associated with a decrease in the degree of its coupling, characterized by a marked decrease in the P/O ratio.

The material described above confirms the views [1, 2] on transition of the mitochondria of the parietal cells during active HCl secretion to a regime of predominantly free oxidation. The mechanism of this transition is based on the creation of a directional flow of electrons over the outer nonphosphorylating membrane of the mitochondria. The connecting link in this case is evidently cytochrome c, the outflow of which into the

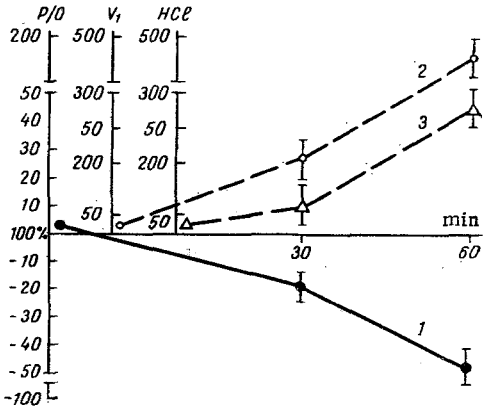

Fig. 2. Ratio between oxidation and phosphorylation in mitochondria of gastric mucosa under conditions of basal and histamine-stimulated (0.04 mg/kg) HCl secretion. Abscissa, time (in min); ordinate, changes in indices studied (in % of initial level). Curves: 1) phosphorylation coefficient (P/O); 2) rate of O_2 utilization (V_1); 3) state of acid-producing function (HCl).

intermembranous space determines the direction of movement of the electrons and is due to the action of factors increasing permeability of the inner mitochondrial membrane (thyroxine and its acetic acid analogues, unsaturated fatty acids). In the course of subsequent contact between the outer mitochondrial membrane and the cytoplasmic membrane the energy of electron transport is utilized directly for translocation of H^+ ions through the cell membrane.

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